Biological synthesis of silver nanoparticles using Aegle marmelos (L.) CORREA EX. SCHULTZ stem

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Abstract There is worldwide interest in silver nanoparticles (AgNPs) synthesized by various chemical reactions for use in applications exploiting their antibacterial activity. The reason for the green synthesis method was employed for the synthesis of silver nanoparticles (AgNPs) in the present study. Unlike chemical method green synthesis method is cost-effective, non-toxic and easy to perform. In this study, Aegle marmelos stem extract was used for the synthesis of AgNPs and the synthesized AgNPs were characterized by UV-Visible spectrometry, Fourier transform infrared (FTIR) spectrometer and Transmission Electron Microscopy (TEM). The prepared AgNPs also evaluated for antibacterial activity against both Gram positive and Gram negative bacteria. The results showed that the synthesized AgNPs exhibited good antibacterial activity than the standard antibiotic Streptomycin.

Keywords – Nano particles, marmrlos, CORREA, Stem,

I. INTRODUCTION

Nano-biotechnology is one of the fastest developing science during the last few yearsand manipulation of particle's structure ranging from approximately 1 to 100 nm in size. It is an interdisciplinary science that connects knowledge of biology, chemistry, physics, engineering and material science. Novel applications of nanoparticles and nanomaterials are growing rapidly on various fronts due to their completely new or enhanced properties based on size, their distribution and morphology. The metallic nanoparticles considered as the most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest for researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains (Khalil et al., 2013). Of the nanoparticles used in the pharmaceutical industry, silver nanoparticles (AgNPs) are one of the important materials in nanomedicine. Silver nanoparticles (Ag NPs) have been used as antibacterial agents for topical application for bacterial skin infections (Mal et al., 2014; Gopinath et al., 2015).There are a variety of methods to synthesize AgNPs including physical and chemical methods. Chemical routes are effective; these methods may suffer from toxicity due to the chemicals used and the difficulty in removing them. Additionally, chemical reagents used in these methods are hazardous to the environment (Nabikhanet al., 2010). To avoid the toxicity of chemicals, green synthesis was developed (Sharma et al., 2009). This method of biosynthesis of metal nanoparticles has been proposed as a cost-effective and environmental friendly way of fabricating these materials. Aegle marmelos (L.) CORREA EX. SCHULTZ species belongs to Rutaceae and is globally distributed across Indo-Malesian region and cultivated in Africa. In India, it is reported to occur in the Sub-Himalayan tracts from Jhelum eastward and also southward to the Central and Southern India. The fruit, leaves and root of this plant is used in the form of powder, juice and decoction to treat diarrhea, sprue, piles, oedema, jaundice, vomiting, obesity, deafness, eye diseases, pediatric diseases, fever and as a rejuvinative.

In the present study, the synthesis of silver nanoparticles using Aegle marmelos stem part was studied and also characterized the synthesized AgNPs by UV-Visible spectrometry, Transmission Electron Microscope (TEM). The AgNPs were also tested for their antibacterial activity against both Gram positive and Gram negative bacteria.

II. MATERIALS AND METHODS

Synthesis of silver nanoparticles (AgNPs)

5 g of finely cut Aegle marmelos stem parts were boiled in 100 ml water for 10 min and filtered to obtain stem extract. The extract of Aegle marmelosstem (5 ml) were mixed with 45 ml of 1 mm silver nitrate ($AgNO_3$) and heated at 60^oC. The colour change was observed indicating the formation of AgNPs. The resulted solution containing AgNPs were centrifuged at 10,000 rpm for 15 min and the precipitate was thoroughly washed with sterile distilled water to get rid of any unwanted impurities. The purified pellet was then dried at 60^o C.

Characterization of synthesized AgNPs

UV-Vis spectral analysis was done by using Shimadzu UV-visible spectrophotometer. Biomolecules responsible for the reduction of silver salt were studied using Fourier transform infrared (FTIR) spectrometer (Thermoscientific Nicolet

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380).Transmission electron microscopy (TEM), operated for the size measurement of synthesized AgNPs.

Antibacterial activity screening of AgNPs

The antibacterial activity was carried out by using agar well diffusion method. Antibacterial activity was tested against some Gram positive bacteria viz, Enterococcus faecalis MTCC 439, Bacillus subtilis MTCC 441, and Streptococcus mutans MTCC 497 and Gram negative bacteria namely Proteus vulgaris MTCC 426, Enterobacter aerogenes MTCC 10208, and Pseudomonas aeruginosa MTCC 1688.

III. RESULTS AND DISCUSSION

UV Studies

The result of the UV-Visible spectrum (Fig.1) and colour change of the solution clearly showed that the synthesis of silver nanoparticles occurred. The peak at 435 nm wave length which corresponds to surface plasmon resonance (SPR) of silver nanoparticles and evidenced that the formation of AgNPs.

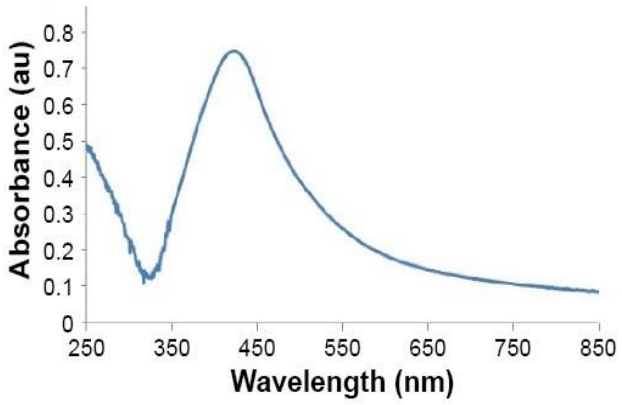

Fig.1. UV-Visible spectrum of Aegle marmelosstem AgNPs

TEM STUDIES

Fig.2 shows the TEM image of AgNPs synthesized by using Aegle marmelosstem extract which predominates with spherical morphologies ranging from 15 to 25 nm with an average size of 20.00 nm. The shape and size of the AgNPs depends upon the reducing agents such as photochemical which are present in the plant extract.

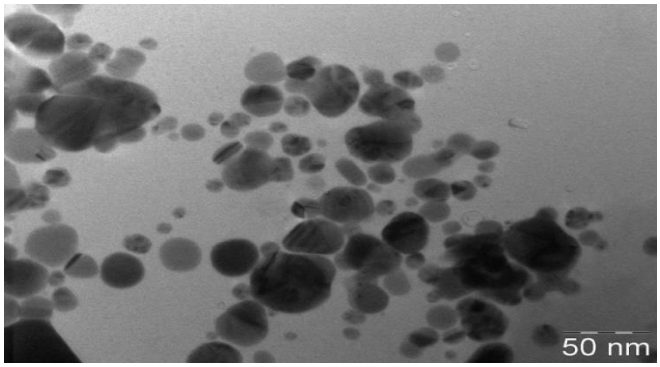

Fig.2 TEM image of *Aegle marmelos*stem AgNPs

IV. ANTIBACTERIAL STUDIES

Antibacterial activity results of AgNPs presented in table-1 and the results showed that all the test organisms including both Gram positive and Gram negative bacteria exhibited larger zone of inhibition than the positive control Streptomycin. Among all the tested bacteria Streptococcus mutans was very sensitive to Aegle marmelosstem AgNPs with 20.00 mm zone of inhibition followed by Bacillus subtilis (19.00 mm), Enterobacter aerogenes (18.33 mm), Enterococcus faecalis (16.00 mm), Proteus vulgaris (14.00 mm) and Pseudomonas aeruginosa (13.33 mm). The antibacterial activity difference between Gram positive and Gram negative bacteria is may be because of the cell wall content of the bacteria. The antibacterial activity of AgNPs can be explained due to the change in the cell membrane permeability or degradation of enzymes in bacteria(Aparajita and Mohan Singh, 2016). There is some literature showing the electrostatic attraction between positively charged nanoparticles and negatively charged bacterial cells (Cao et al., 2001) and they are suggested to be most suitable bactericidal agent (Wright et al., 1999; Matthew et al., 2009).

Table 1. Antibacterial activity of Aegle marmelosstem AgNPs

Test organism	Diameter of inhibition zone of sample (mm)	Diameter of inhibition zone of Streptomycin (mm)
Enterococcus faecalis MTCC 439	16.00±0.00	7.00±0.00
Streptococcus mutans MTCC 497	20.00±0.00	8.00±0.00
Bacillus subtilis MTCC 441	19.00±0.00	7.00±0.00
Enterobacter aerogenes MTCC 10208	18.33±0.57	--
Proteus vulgaris MTCC 426	14.00±0.00	7.00±0.00
Pseudomonas aeruginosaMTCC 1688	13.33±0.00	7.00±0.00

Values given are the means of triplicates ± Standard deviation

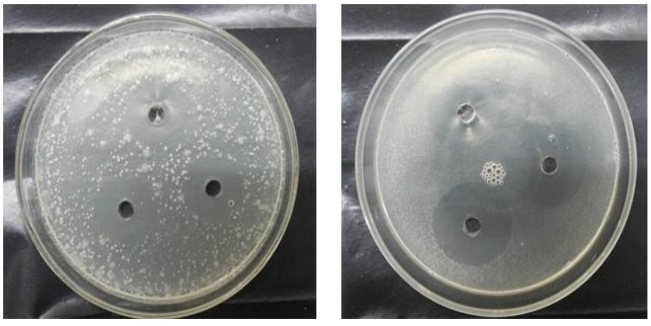

Fig.3 Zone of inhibition images of *Aegle marmelos*stem AgNPs

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